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A DEUTEROSTOME-LIKE NEPHRIDIUM IN THE MITRARIA LARVA OF *OWENIA FUSIFORMIS* (POLYCHAETA, ANNELIDA)

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Abstract

The mitraria larva of *O. fusiformis* possesses a pair of nephridia which are not typical polychaete larval protonephridia, rather they resemble the pore canal-hydropore complex of deuterostome larvae. An ultrastructural analysis of the nephridium reveals a small body cavity, lined by monociliated podocytes, which opens to the exterior by a monociliated nephridioduct. Extracellular matrix between podocyte pedicels forms the filtration surface and podocyte and nephridioduct cilia produce the pressure gradient driving filtration. Therefore, each nephridium is considered a giant protonephridium. Although this organization is unique to *Owenia* among the annelids, it is identical ultrastructurally to the hydropore, pore canal, and adjoining coelom of deuterostome larvae, which is also a nephridium. It is concluded that this cytological similarity between the nephridia of the mitraria and deuterostome larvae reflects a cellular homology which has a wide distribution and is of limited usefulness in the recognition of relationships. The anatomical differences between them suggest they are, at best, serially homologous organs. Mitraria nephridia compare more favorably to the typical protonephridia (head kidneys) of polychaete trochophores and larval protonephridia of phoronids. Data support the view that *Owenia* is primitive among the polychaetes and suggest that the mitraria nephridium represents the plesiomorphic design of protonephridium within the Polychaeta.

Introduction

A recent study testing the generality of a model explaining nephridial diversity in the Metazoa (see Ruppert and Smith, 1985; in prep.) reported that the pore canal-hydropore complex in bipinnaria larvae of echinoderms and tornaria larvae of hemichordates are functional nephridia (Ruppert and Balser, 1986). Our attention was subsequently drawn to the larval nephridium of the polychaete *Owenia fusiformis* by Wilson's (1932) classic study on the mitraria larva. His data indicate that the nephridium is not a typical polychaete larval protonephridium: monociliated or multiciliated terminal cells joined to a nephridioduct which opens on the larval hyposphere (Pemerl, 1965; Holborow, 1971; Wessing and Polenz, 1974; Heimler, 1981; Smith and Ruppert, in press). Instead, each nephridioduct joins a small sac-like cavity that attaches to the episphere by a muscle band. These nephridia resemble the pore canal-hydropore complex of deuterostome larvae.

Two theories are used to explain the evolution of the annelids. One proposes that the segmental coelom evolved directly from the acoelomate organization (Goodrich, 1945; Clark, 1964) and predicts that primitive characters will be shared by annelids

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and species of acoelomate taxa. The other proposes that the segmental coelom evolved from coelomate organization (Remane, 1963, 1967; Siewing, 1976, 1980, 1981) and predicts that primitive characters will be shared uniquely by annelids and species of coelomate taxa. The explanatory value of either theory depends on the recognition of homologous structures between annelids and other phyla of animals.

The organization of *Owenia fusiformis* has figured prominently in recent discussions of the evolution of annelids from pre-annelidan coelomate stock (Gardiner, 1978, 1979). A comparative structural and ultrastructural analysis of the tentacles and body wall of *Owenia* identified several primitive structures shared with lophophorates, echinoderms, and hemichordates ("archicoelomates" of European literature; Gardiner, 1978, 1979; Gardiner and Rieger, 1980).

An ultrastructural investigation of the organization of the larval nephridium of *O. fusiformis* was undertaken to compare it with the pore canal-hydropore complex (nephridium) of deuterostome larvae as described by Ruppert and Balser (1986). We present evidence that the nephridium of the mitraria is morphologically identical to the larval deuterostome nephridium and discuss both its function and relationship to the nephridia of deuterostome and non-deuterostome larvae.

Materials and Methods

Mitraria larvae of *Owenia fusiformis* delle Chiaje were obtained from plankton tows off of Pawley's Island, South Carolina. Mitraria larvae were identified as belonging to *Owenia* rather than to *Myriochele,* the other genus of the Oweniidae whose larvae are found in the plankton off of this coastline, by the number of cilia per cell. Cells of the mitraria of *Owenia* are monociliated, whereas cells of the mitraria of *Myriochele* are milticiliated. Specimens were relaxed in magnesium chloride isotonic to seawater and fixed for 48 h at room temperature in 2.5% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer (pH 7.6) and 0.34 *M* sodium chloride. They were post-fixed for 1 h at room temperature in 1% osmium tetroxide in 0.34 *M* sodium chloride and 0.2 *M* Millonig's phosphate buffer (pH 7.6). Following post-fixation, specimens were dehydrated in ethanol, transferred through two changes of propylene oxide, and embedded in Polybed 812 (Polysciences, Inc., Warrington, Pennsylvania). One micrometer and thin sections were cut with diamond knives on a LKB Ultrotome Nova. One micrometer sections were stained with methylene blue. Thin sections were collected on bare copper hexagonal mesh grids, stained with alcoholic uranyl acetate and lead citrate, and examined with a Philips EM 300 transmission electron microscope. The terminology used for larval structures and their spatial orientation is based upon Wilson (1932).

Results

The mitraria larva of *Owenia fusiformis* possesses a single pair of nephridia. Each nephridium is situated in the blastocoel, lateral to the setal sacs (Figs. 1, 2A). Both nephridia attach to the larval episphere by the dorsal levator muscles and lie directly against the ventral epidermis of the hyposphere (Figs. 1, 2A). Each nephridium is approximately 40 μm in length and it ranges in height from approximately 12 μm proximally, at the point of attachment of the dorsal levator muscles, to approximately 6.5 μm distally, where it opens into the nephridioduct. The tubular nephridioduct extends anteriorly from each nephridium and opens to the exterior through the first segment of the developing worm rudiment. It averages 30 μm in length and 3.0 μm in diameter.

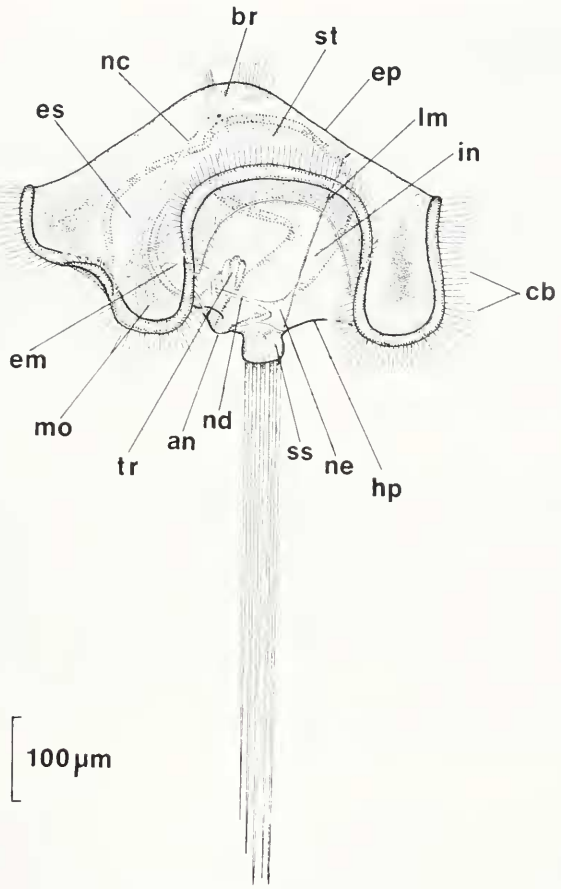

FIGURE 1. Diagram of the mitraria larva of *Owenia fusiformis* (after Wilson, 1932). an, anus; br, cerebral ganglion; cb, prototrochal ciliary band; em, esophageal muscle; ep, episphere; es, esophagus; hp, hyposphere; in, intestine; lm, dorsal levator muscles; mo, mouth; nc, circumesophageal nerve cord; nd, nephridioduct; ne, nephridium; ss, setal sac; st, stomach; tr, trunk rudiment.

Transmission electron microscopic observations indicate that each nephridial sac is lined by monociliated podocytes which lie on a basal extracellular matrix (ECM), averaging 90 nm in thickness (Figs. 2B–D; 3A). At the point of origin of the dorsal levator muscles from the nephridium, the nephridial lumen is lined by both podocytes and myocytes which are continuous with the levator muscle (Fig. 2B). Therefore, the levator muscle is a modified part of the nephridial lining as is the levator muscle in the tornaria larvae of hemichordates (Morgan, 1894; Ruppert and Balser, 1986). Each podocyte consists of a cuboidal perikaryon, 1.7–3.5 μm high, which bulges into the nephridial cavity (Fig. 2B–D), and attenuated, lateral foot processes (pedicels). Filtration slits between adjacent pedicels are 40–70 nm in diameter and lack diaphragms (Fig. 2D). Adhaerens junctions join the lateral surfaces of adjacent podocytes (Fig. 2E inset). The luminal surface of each perikaryon bears a single cilium and filiform microvilli. The cilium, which is oriented towards the duct, arises from a

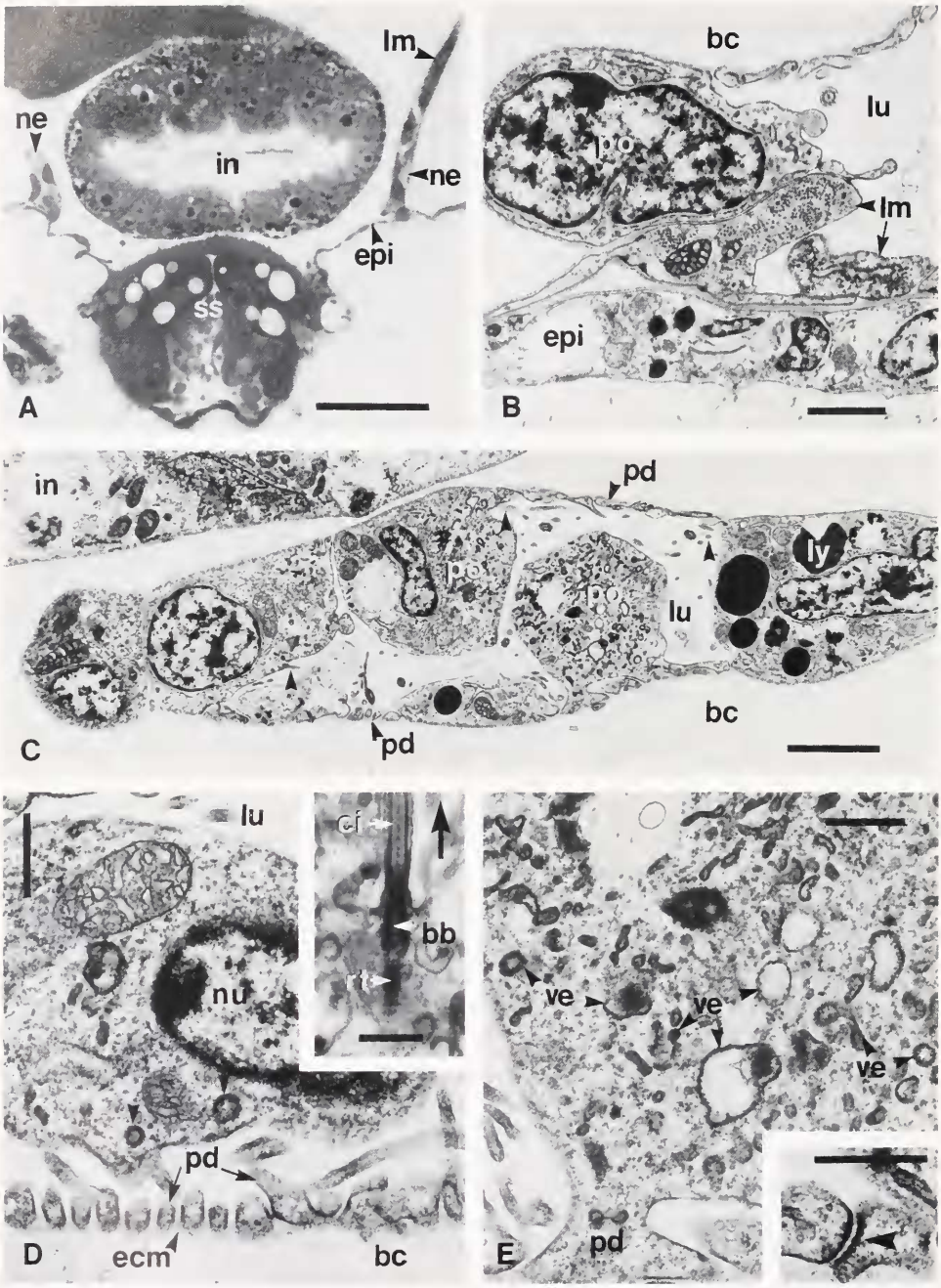

FIGURE 2. Nephridium of mitraria larva of *Owenia fusiformis*. A. Light micrograph of an oblique frontal section through a mitraria larva showing a pair of nephridia (ne) situated within blastocoel of hyposphere. Each nephridium rests on the epidermis (epi) of hyposphere. Note origin of dorsal levator muscle (lm) from nephridium situated on the right. Scale = 20 μm. B. Transmission electron micrograph (TEM) of a longitudinal section through proximal most region of nephridium showing relationship of nephridial podocytes (po) to dorsal levator muscle (lm). The nephridial lumen is lined by both podocytes

shallow pit and has an accessory centriole and caudal rootlet at its base (Fig. 2D inset). The rootlet is oriented towards the duct (Fig. 2D inset). Smooth and coated endocytotic pits occur along the apical and lateral plasmalemmas of each cell body. Perinuclear endocytotic vesicles, endosomes, lysosome-like bodies, small Golgi complexes, mitochondria, and a few RER cisternae occur within the cytoplasm of each cell (Figs. 2C–E, 3A).

The nephridioduct consists of squamous monociliated cells, approximately 3.5 μm in height at the perikarya and 1.9 μm at their lateral margins. The duct cells rest on a basal ECM, 16–30 nm in thickness, (Fig. 3B) and adjacent cells are joined by lateral adhaerens junctions. Each duct cell wraps around itself and encloses an extracellular lumen, which is approximately 0.8 μm in diameter (Fig. 3B–D). An adhaerens junction joins the apposed surfaces of each doughnut-shaped cell (Fig. 3B). Each perikaryon bulges slightly into the lumen and bears a single subapical cilium and long filiform microvilli (Fig. 3B). The cilium emerges from a shallow pit, and an accessory centriole and caudal rootlet occur at its base (Fig. 3C). The ciliary rootlet, like the cilium, is oriented towards the nephridiopore (Fig. 3C). A few scattered smooth and coated endocytotic pits occur along the apical plasmalemma of the duct cells. Perinuclear endocytotic vesicles, endosomes, lysosome-like bodies, mitochondria, small Golgi complexes, and RER cisternae occur in each cell (Fig. 3B–D).

Discussion

Polychaete larvae possess protonephridia with either monociliated (Goodrich, 1945; Holborow, 1971; Smith and Ruppert, in press) or multiciliated (Goodrich, 1945; Pemerl, 1965; Wessing and Polenz, 1973; Heimler, 1981; Smith and Ruppert, in press) terminal cells. The results of this study reveal that the nephridium of the mitraria larva of *O. fusiformis* is not a typical protonephridium, rather it is a small body cavity lined by monociliated podocytes which opens to the exterior by a ciliated duct. Although this organization is unique to *Owenia* among the annelids, it is identical to that of the hydropore, pore canal, and adjoining coelom of deuterostome larvae which also is a nephridium (Ruppert and Balser, 1986).

Two dominant types of nephridia are recognized in aquatic invertebrates, protonephridia and metanephridia. Although typically defined solely on their morphology and germ layer origin (see Goodrich, 1945; Wilson and Webster, 1974), they can also be defined functionally. A protonephridium is an excretory organ where cilia-mediated filtration occurs on the nephridial wall and a metanephridium is an excretory organ where muscle-mediated filtration occurs on blood vessels or their analogs

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and myocytes of the levator muscle in this region. Scale = 1 μm. C. TEM of a parasagittal section through nephridial sac. Note podocytes (po) lining nephridial lumen (lu) and pedicles (pd) and microvilli (arrowheads) extending from podocytes. Scale = 2 μm. D. TEM of a nephridial podocyte showing its pedicels (pd) resting on the underlying basal extracellular matrix (ecm) which forms the filtration barrier. Note perinuclear endocytotic vesicles (arrowheads) within its cytoplasm. Scale = 0.5 μm. Inset: TEM of single cilium (ci) of a podocyte and its associated basal body (bb) and rootlet (rt). An accessory centriole, although present, is not shown in this section. Arrow indicates direction of nephridiopore. Scale = 0.5 μm. E. TEM of a nephridial podocyte showing numerous endocytotic vesicles (ve) situated within its cytoplasm. Scale = 0.5 μm. Inset: Adhaerens junction (arrowhead) between two adjacent podocytes. Scale = 0.5 μm. bb, basal body; bc, blastocoel; ci, cilium; ecm, basal extracellular matrix; epi, epidermis of hyposphere; in, intestine; lm, dorsal levator muscle; lu, nephridial lumen; ly, lysosome-like bodies; ne, nephridium; nu, nucleus; pd, pedicel; po, podocyte; ss, setal sac; ve, endocytotic vesicles.

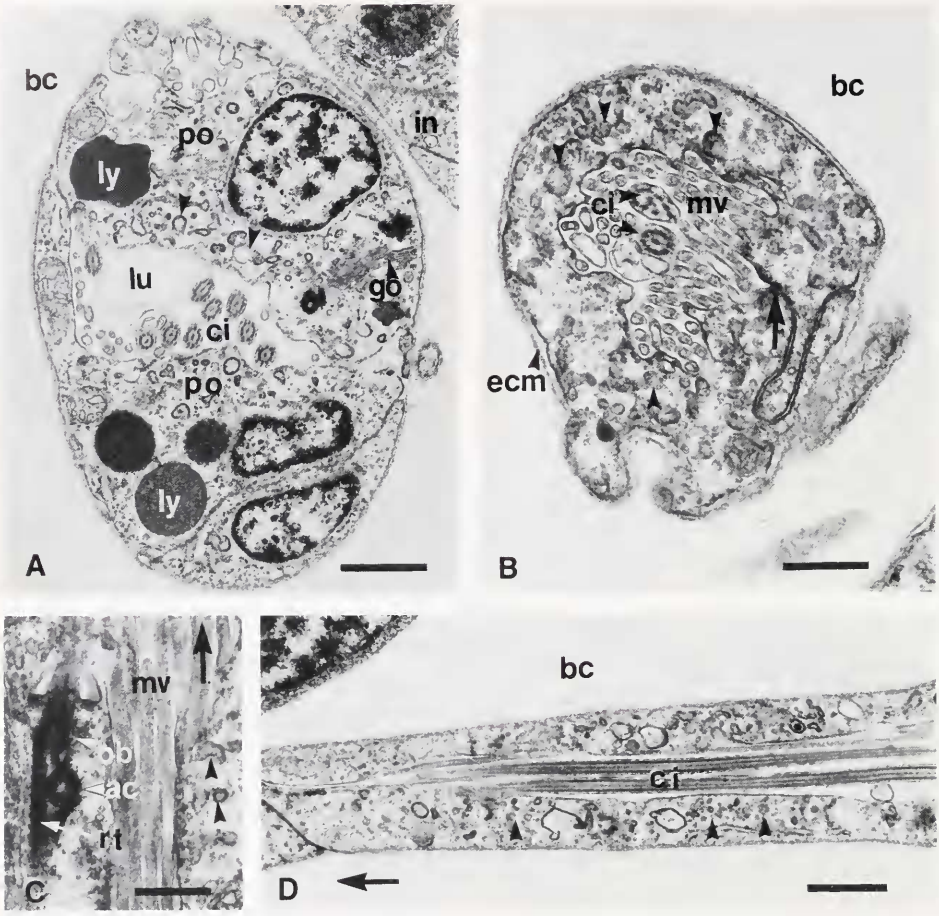

FIGURE 3. TEMs of nephridium and nephridioduct of mitraria larva of *Owenia fusiformis*. A. Transverse section through distal most portion of nephridium near point of union with nephridioduct. Note lysosome-like bodies (ly) within cytoplasm of podocytes. Scale = 1 μm. B. Transverse section through medial portion of nephridioduct. Note adhaerens junction (arrow) between apposed surfaces of doughnut-shaped cell and numerous endocytotic vesicles (arrowheads) within its cytoplasm. Cilia (ci) situated within its lumen extend from duct cells. Scale = 0.5 μm. C. Ciliary basal body (bb), rootlet (rt), and accessory centriole (ac) of a nephridioduct cell. Note microvilli (mv) within duct lumen and endocytotic vesicles (arrowheads) within cytoplasm of duct cell. Arrow indicates direction of nephridiopore. Scale = 0.5 μm. D. Parasagittal section through distal most portion of nephridioduct. Note endocytotic vesicles (arrowheads) within duct cell cytoplasm. Arrow indicates direction of nephridiopore. Scale = 1 μm. ac, acessory centriole; bb, basal body; bc, blastocoel; ci, cilia; ecm, basal extracellular matrix; in, intestine; lu, lumen of nephridioduct; ly, lysosome-like body; mv, microvilli; po, podocyte; rt, rootlet.

(Ruppert and Smith, 1985; in prep.). Based on these definitions, each mitraria nephridium is a giant protonephridium. Presumably the ECM between the podocyte pedicels forms the filtration surface while the podocyte and duct cilia produce the filtration pressure gradient. Blastocoelic fluid may be ultrafiltered across the ECM between the pedicels. Numerous endocytotic vesicles in the podocyte perikarya suggest that modification of the ultrafiltrate occurs within the nephridium. Additional

modification may occur along the nephridioduct before the fluid is discharged at the nephridiopore. As suggested by Ruppert and Balser (1986) for nephridia of deuterostome larvae, osmotic fluid recovery across the body wall of the mitraria may be established by proteins in the blastocoelic fluid.

The pore canal-hydropore complexes contribute during metamorphosis to the metanephridial systems of adult hemichordates and possibly asteroid echinoderms (Ruppert and Balser, 1986), whereas the nephridium of the mitraria undergoes histolysis (Wilson, 1932) and is superseded by a pair of metanephridia in the sixth adult segment (setiger five).

Ultrastructurally, the mitraria nephridium is identical to those of larval enteropneusts and asteroids, but are they homologs or analogs? Consideration must be given to comparative larval anatomy and functional constraints on nephridial design in larval body organization to provide a tentative answer to this question. We have previously discussed the correlation of cilia-driven filtration nephridia (protonephridia) with the absence of blood vessels and the physical basis for the lack of circulatory systems in small animals, such as many larvae (Ruppert and Smith, 1985; in prep.). On the basis of functional considerations, protonephridia are expected in small animals.

Comparison of the mitraria nephridium with that of a tornaria, with which it seems directly comparable, reveals histological similarities and anatomical dissimilarities. The nephridia of both larvae are lined by an epithelium composed of monociliated podocytes and myocytes. The myocytes form an apical muscle band that traverses the blastocoel and inserts on the episphere, but the point of insertion is different in the two larvae. In the mitraria, insertion is posterior to the cerebral ganglion, whereas in the tornaria it is on the ganglion. Moreover, the mitraria nephridia are situated postorally and the nephridioducts open to the exterior through the first pair of segmental somites, whereas the nephridium and its dorsal duct in the tornaria are situtated preorally. In addition, the mitraria nephridia are paired while those of the tornaria are unpaired, although paired ducts, apparently atavisms, occur frequently in laboratory reared larvae (Gemmill, 1914; Ruppert, unpub. obs.).

We conclude that the striking cytological and histological similarities between the mitraria nephridia and those of asteroid and enteropneust larvae reflect a cellular homology, the common possession of monociliated podocytes. Like myocytes or neurons which also occur widely in metazoans, podocytes *per se* are of limited usefulness in the recognition of relationships. On the other hand, the anatomical differences cited above suggest mitraria nephridia, as organs, may not be homologous to those of hemichordates and echinoderms; at best they might be serial homologs. However, this does not weaken the archicoelomate affinities of *Owenia* because the mitraria nephridia compare favorably to the monociliated larval protonephridia of phoronids. Although Wilson (1932) rejected the correspondence of mitraria nephridia with typical protonephridia (head kidneys) of other polychaete trochophores, we suggest that the two are homologous and that the differences encountered between them may be explained in terms of larval body size (Ruppert and Smith, in prep.).

Owenia is viewed as being primitive within the extant Polychaeta because it possesses several characters which are believed to be plesiomorphic within the Metazoa. These include an unspecialized monociliated epidermis (Gardiner, 1978, 1979), a basiepidermal nervous system (Gardiner, 1978, 1979), and muscle cells with a single rudimentary cilium (Gardiner and Rieger, 1980). Do the larval nephridia further corroborate the view that *Owenia* is primitive among the polychaetes?

Polychaetes are, in basic structural design, vermiform, soft-bodied, segmented

coelomates. Based on recent discussions of function, this implies that adult polychaetes rely on a hydrostatic skeleton for locomotion (Clark, 1964), a blood vascular system for a through-flow internal transport system (Ruppert and Carle, 1983), and a metanephridial system for excretion (Ruppert and Smith, in prep.). If an unsegmented small-bodied larva, such as a trochophore, is included in the basic polychaete life cycle, then on functional grounds it should have a protonephridial system (Ruppert and Smith, in prep.). Therefore, it is possible that the stem species of the Polychaeta had a larval protonephridium and an adult metanephridium. In that case, neither the protonephridium nor the metanephridium is primitive to the other, rather they are co-primitive organs in polychaetes, each of which may be modified in particular lineages (Smith and Ruppert, in press).

O. fusiformis possesses the nephridial design postulated above for the adult and larva of the polychaete stem species. The segmented adult of *O. fusiformis* possesses a blood vascular system and metanephridia and the mitraria larva possesses a protonephridial system. Both the metanephridia of the adult and the protonephridia of the mitraria are composed of monociliated cells. Rieger (1976) has shown that the presence of an epithelium composed of monociliated cells is a plesiomorphic feature within the Metazoa. This suggests that *O. fusiformis* has preserved the original nephridial designs of the adult (a monociliated metanephridial duct) and the larva (a protonephridium composed of monociliated podocytes) of the polychaete stem species, thereby corroborating *Owenia's* position as the most primitive of the extant polychaetes.

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